Carbohydrate Research, 55 (1977) 3–10

STRUCTURAL FEATURES OF THE GUM EXUDATES FROM SOME *Acacia* SPECIES OF THE SERIES *Phyllodineae* AND *Botryocephalae**

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(Received July 9th, 1976; accepted for publication, October 15th, 1976)

ABSTRACT

Smith degradation of each of the polydisperse, gum polysaccharides from *Acacia pycnantha*, *A. difformis*, *A. filicifolia*, and *A. podalyriaefolia*, for which molecular-weight distributions have been measured by gel-permeation chromatography, gives, in good yield, a methanol-insoluble polysaccharide that shows a single peak on examination by this technique. The molecular weights of the Smith-degraded polysaccharides are close to the values expected if the respective gum polysaccharides, on losing periodate-vulnerable, peripheral sugar residues, were to be split also at regular intervals between the otherwise (1→3)-linked D-galactose chains. The structures of these gums, which are similar in many respects, conform to the pattern shown recently to occur in the gum of *A. baileyana*.

INTRODUCTION

Since the technique of periodate oxidation, borohydride reduction, and controlled acid hydrolysis was first applied by F. Smith and his co-workers to gum arabic[1], *Acacia* gum samples from numerous species have been shown, without exception, to contain an internal core of β-(1→3)-linked D-galactopyranose residues[2]. Sequential Smith-degradations[3] have given additional information about the nature of peripheral sugar groups, and measurements of the molecular weights of the periodate-resistant portions of the polysaccharides have been of value in the subclassification of gum-forming *Acacia* species[4]. Recently, Smith-degradation procedures have shown that certain low molecular-weight *Acacia* gums, although polymolecular, contain blocks of galactose residues that are not attacked by periodate but which are separated at regular intervals by sugar residues that are; the consequence is that suitably controlled, acid hydrolysis of the oxidised, and subsequently reduced gum yields a product that shows either a single peak on gel chromatography[5], or

*Dedicated to the memory of Professor J. K. N. Jones, F.R.S.

two or more peaks that bear a simple molecular-weight relationship to one another[6].

Accordingly, further examples of *Acacia* gums have been examined in order to test whether the structural pattern that has emerged is of general applicability. It was of particular interest to apply Smith degradation to the gum of *A. pycnantha* (Series *Phyllodineae*) because of the detailed structural model that was available through the extensive researches of other workers[7], including Barry degradation[8] of the oxidised polysaccharide. The gums of *A. difformis* (Series *Phyllodineae*)[9] and *A. filicifolia* (Series *Botryocephalae*)[10] were also selected for examination because published analytical data for these gums indicate close similarity to the gums from these Series studied earlier[5,6].

EXPERIMENTAL

Sources of gum specimens. — Samples of the gums of *Acacia difformis* and *A. filicifolia*, collected in New South Wales, Australia in January, 1970 and February, 1970, respectively, were made available through the courtesy of Dr. D. M. W. Anderson (Edinburgh), who has published[9,10] analytical data for these specimens. The samples of *A. pycnantha* gum used in these studies were collected near Stellenbosch, Cape Province, South Africa in November, 1974 and October, 1975, and several different samples of *A. podalyriaefolia* gum, originating in Johannesburg, Port Elizabeth, and Cape Town (South Africa) were collected in July, 1964, July, 1975 and May, 1976, respectively.

Purification of gum samples. — Small samples of the gums, as collected from *Acacia* stems or branches, were examined by gel-permeation chromatography, but most of the experimental work was performed on bulked material that had been recovered by precipitation with ethanol (4 volumes) from clear, aqueous solutions of these gums. The precipitated polysaccharides were washed with ethanol and ether, redissolved in water, and freeze-dried.

General experimental conditions. — Optical rotations were measured at 20° on a Perkin–Elmer 141 polarimeter, by using aqueous solutions in the concentration range 0.5–1% unless otherwise specified. The solvent systems used in paper chromatography were (*A*) 4:1:5 1-butanol–ethanol–water (upper layer), (*B*) 2:1:1 1-butanol–acetic acid–water, and (*C*) 8:2:1 ethyl acetate–pyridine–water. Gel-permeation chromatography was conducted on Bio-Gel P-300 or P-10 as appropriate, with M sodium chloride as eluent[3,11]. Proportions of neutral sugars were determined by g.l.c. of the derived alditol acetates[12], following hydrolysis of the polysaccharides and their degradation products in sealed tubes, under nitrogen, with 2M trifluoroacetic acid for 8 h at 100°; the aqueous acid was normally removed by freeze-drying. Periodate consumption was monitored by the arsenite method[13].

Methylation analysis. — After methylation of the gums by the Hakomori[14] and Purdie[15] (2–4 times) procedures, the mixtures of methylated sugars produced on hydrolysis (trifluoroacetic acid as already described, for 18 h) were analysed by g.l.c. of the trimethylsilyl ethers of the derived alditols[16].

Smith degradation. — Oxidation of each polysaccharide with aqueous sodium metaperiodate[6] was terminated by addition of the calculated quantity of barium acetate, and the centrifugate was reduced with sodium borohydride. The reduced, oxidised polysaccharides, recovered as previously described[5], were submitted to mild hydrolysis in M trifluoracetic acid for periods ranging from 54 to 120 h at room temperature. The decision to terminate the degradation, by freeze-drying the acidic solution, was governed in each case by the extent of diminution in $\bar{M}_w$ of the polysaccharide and the degree of polymolecularity of the products as shown by gel-permeation chromatography on Bio-Gel P-10. Hydrolysis was stopped when the molecular-weight distribution of the resultant, degraded polysaccharide remained constant over a 24-h period, or longer, in contact with M trifluoroacetic acid. After Smith degradation had been carried to completion, all of the gums yielded methanol-insoluble, degraded polysaccharides that were monodisperse and did not decrease in molecular weight on further contact with M trifluoroacetic acid at room temperature. The degraded polysaccharides (after paper chromatography of their hydrolysates had demonstrated the absence of uronic acid) were then treated in aqueous solution with mixed ion-exchange resins and freeze-dried. The methanol-soluble fractions of the Smith-degradation products were shown by paper chromatography to consist largely of polyol (glycerol, mainly), together with low molecular-weight glycosides.

RESULTS AND DISCUSSION

The gums of *A. pycnantha*, *A. difformis*, and *A. filicifolia* show a close resemblance, both in the properties listed in Table I and in their structural components as indicated by methylation analysis (Table II), to the gums of *A. podalyriaefolia*, *A. elata*, and *A. baileyana* previously examined[5,6]. Partial-hydrolysis experiments reported elsewhere[17] have confirmed that *A. pycnantha* gum has the same general structural features as the last three gums just named[5,18,19], notably acid-labile rhamnose, arabinose and galactose residues in short branches attached to a galactan core that ultimately breaks down to a series of neutral oligosaccharides containing β-(1→3)-linked D-galactose residues and, in small proportions, the aldobiouronic acid 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. Paper chromatography (solvents *B* and *C*) of partial hydrolysates (0.01M trifluoroacetic acid, 48 h, 100°) shows that the gums of *A. difformis* and *A. filicifolia* also yield these products. The approach of using Smith degradation to ascertain the distribution of the β-(1→3)-linked D-galactose residues in the polysaccharide molecule has proved illuminating in previous studies[3–6], particularly with *A. baileyana* gum[5] which yields, after a single Smith degradation, a monodisperse, degraded polysaccharide (molecular weight 2500) in which (1→3)-linked D-galactose residues preponderate, although a small proportion of constituent arabinose remains. There is strong evidence relating the production of this single, degraded polysaccharide to the occurrence of repeating sub-units having molecular weight 4600 in the molecule of the intact polysaccharide.

TABLE I

PROPERTIES OF GUM POLYSACCHARIDES FROM *Acacia pycnantha*, *A. diffformis*, AND *A. filicifolia*

Gum of	A. pycnantha	A. diffformis	A. filicifolia
$[\alpha]_D$ (degrees)	−7	−9	+7
$\overline{M}_w$[a]	12000	16000	13000
Molecular-weight distribution[a,b]	31600(2); 24000(6); 17800(18); 12000(36); 8000(17); 6000(21)	34000(3); 28200(8); 23500(14); 15000(37); 12000(10); 10000(15); 7000(13)	28200(2); 21400(8); 16800(16); 14000(9); 10800(40); 8400(25)
Equivalent weight	3700 (ref. 7)	3400 (ref. 9)	4300 (ref. 10)
Hence uronic acid (mol %)	5	5	4
Proportions of neutral sugars (mol %)			
Galactose	67	73	81
Arabinose	26	20	14
Rhamnose	2	2	1
Periodate consumption ($mmol.g^{-1}$)	5.7	6.7	6.2

[a]Bio-Gel P-300. [b]Molecular weights corresponding to peaks in gel chromatograms; relative proportions by weight in parentheses.

TABLE II

METHYLATION ANALYSIS OF *A. pycnantha*, *A. diffformis*, AND *A. filicifolia* GUMS[a]

Partially methylated sugar (positions of O-methyl groups given by locants)	*Molar proportions*		
	A. pycnantha *gum*[b]	A. diffformis *gum*[c]	A. filicifolia *gum*[c]
2,3,5-Ara	18	15	11
2,5-Ara 3,5-Ara 2,3,4-Rha	5	3	3
2,3-Ara 3,4-Ara	1	3	2
2,3,4,6-Gal	20	20	28
2,4,6-Gal	3	3	5
2,3,6-Gal	1	3	2
2,3,4-Gal	7	9	2
2,6-Gal	4	3	1
2,4-Gal	30	33	41
2-Gal	6	3	1

[a]$[\alpha]_D$ for chloroform solutions of the methylated gums, −50 (Ref. 22), −45, and −47°, respectively. [b]Compare ref. 22, where the proportions given are similar except for end-group D-galactopyranose (found to be higher by assay based upon methyl glycosides). [c]Values given were corroborated by g.l.c. analysis of the alditol acetates; compare ref. 23, where methyl glycosides were employed.

The data presented in Table III show clearly that the gums of *A. pycnantha*, *A. difformis*, and *A. filicifolia* also yield, after one Smith degradation, monodisperse degraded polysaccharides that are essentially galactans, containing arabinose as a minor constituent. The specific rotations of these three degraded gums and the amounts of periodate consumed by the whole gums (Table I) are similar to those found for gums of this type studied earlier. Monodispersity of the degraded polysaccharides is indicated by both gel-permeation chromatography and ultracentrifugation[20], and the molecular-weight values obtained by these two independent procedures are in good agreement (Table III). T.l.c. on plates coated with Kieselguhr G [solvent system[21] 4:4:2 (v/v) 2-propanol–acetone–M lactic acid, to which 3–4 parts by volume of water had been added] of each of these three Smith-degraded polysaccharides and that from *A. baileyana* gum gave single spots (R_F ~0.8).

TABLE III

PROPERTIES OF METHANOL-INSOLUBLE PRODUCTS OF SMITH DEGRADATION OF GUMS

Smith-degraded gum of:	A. pycnantha	A. difformis	A. filicifolia
$[\alpha]_D$ (degrees)	+28	+32	+29
Galactose:arabinose ratio	95:5	97:3	97:3
Methylated derivative: $[\alpha]_D$ in $CHCl_3$ (degrees)	−12	−6	−5
Molecular weight of Smith-degraded polysaccharide	3500[a] 4100[b]	3600[a] 3800[b]	2100[a] 2100[b]
Calculated molecular weight of corresponding block in whole gum	6600	6800	4200

[a]From gel-permeation chromatography (Bio-Gel P-10). [b]From sedimentation and diffusion measurements[20]. The predominance of (1→3) linkages in all three Smith-degradation products was proved by methylation analysis.

During the Smith-degradation procedure, the mild acid-hydrolysis step that ultimately yielded these monodisperse, degraded polysaccharides was monitored by gel-permeation chromatography; this method proved more sensitive than others used previously[6]. Before the degradation was complete, gel-permeation chromatography (Bio-Gel P-10) of all of these gums showed the presence of components having molecular weights that approximated to multiples (2 and 3 times) of the molecular weights of the final products (see, for example, Fig. 1). This observation indicates a periodicity in the galactan skeletons of the gums, with acetal linkages between the blocks of sugar residues that survive the periodate oxidation; these linkages are separated at regular intervals, and are broken relatively slowly during the hydrolysis step. Precise definition of the length of the galactan chains is not yet possible, although the narrowness of the elution band on gel-permeation chromatography of smaller samples than that illustrated in Fig. 1, and preliminary fractionation experiments with high-pressure liquid chromatography (eluting with 2-propanol–water from a Partisil 10 column), support the view that the range cannot be large.

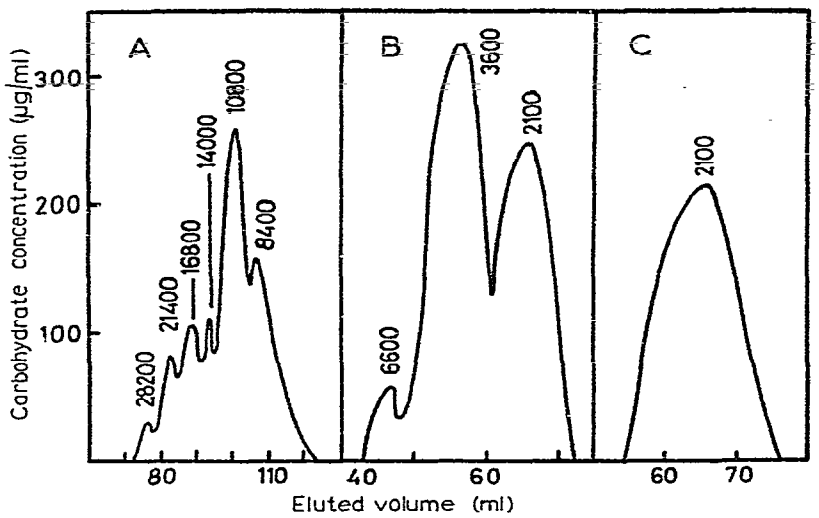

Fig. 1. Gel-permeation chromatograms showing progress of Smith degradation of *A. filicifolia* gum (A). Reduced, oxidised gum after hydrolysis in M trifluoroacetic acid for 54 h at room temperature (B) and for 92 h (C). Further exposure to acid for 24 h left the pattern (C) unchanged. Eluent: M sodium chloride. Columns: (A) Bio-Gel P-300, 85 × 1.5 cm; (B, C) Bio-Gel P-10, 55 × 1.5 cm.

Allowing for the proportion of sugar residues removed by the Smith degradation (end-groups, and some arabinose and galactose present as glycosides in the methanol-soluble material produced) permits calculation, as in the case of *A. baileyana* gum[5], of the molecular weights of the blocks of sugar residues in the intact polysaccharides from these gums that give rise to the degraded polysaccharides (see Table III). Scrutiny of the molecular-weight distributions of the gums (Table I) shows that most of the components present have molecular weights that are approximately multiples of the calculated values for the sub-units in the gum.

The gum of *A. podalyriaefolia* was previously reported[6] to yield a polymolecular, Smith-degraded polysaccharide, but further contact of this product with 0.01M trifluoracetic acid for 65 h at room temperature results in the disappearance of (minor) components having molecular weights 8400 and 5000, yielding a monodisperse polysaccharide of molecular weight 2100. This corresponds to a sub-unit, in the gum, of molecular weight approximately 4000. The various samples of the gum examined have been found to vary considerably in $\overline{M}_w$ (as reported[4] for some gums from other *Acacia* species), with values ranging from 9500 to 32000, even among samples taken from different branches of the same young tree. However, this variation was due to the presence, in differing proportions in the different samples, of components having molecular weights of the order of 4000, 8000, 16000, and 32000 in all cases. These are, once again, integral multiples of the molecular weight of the sub-unit indicated by Smith degradation.

It has thus been shown that five polymolecular arabinogalactan gums, having relatively low $\overline{M}_w$, from *Acacia* species of the series *Phyllodineae* (*A. podalyriaefolia*,

A. pycnantha, and *A. difformis*) and *Botryocephalae* (*A. baileyana* and *A. filicifolia*) give a monodisperse, degraded polysaccharide after a single Smith degradation. A notable exception is the otherwise similar gum of *A. elata* (series *Botryocephalae*), which yields two Smith-degraded polysaccharides, in nearly equal proportions by weight, having molecular weights 2100 and 4400, respectively[6]. Neither of these products undergoes any diminution in molecular weight when treated with M trifluoroacetic acid for up to 83 h at room temperature, and therefore neither can be ascribed to the result of incomplete degradation. In the five examples cited here, a sugar is present, between blocks of (1→3)-linked galactose residues, so linked as to be vulnerable to attack by periodate and thus to very mild acid hydrolysis in the resulting oxidised, and subsequently reduced polysaccharide. That the polysaccharide itself is resistant to such mild hydrolysis has been demonstrated by gel-permeation chromatography of a sample of *A. pycnantha* gum that had been kept in M trifluoracetic acid for 144 h at room temperature; although paper chromatography showed the release of some arabinose, there was no discernible change in $\overline{M}_w$ and therefore no cleavage of glycosidic bonds in the polysaccharide chains under these conditions. The nature of the inter-sugar linkages between the (1→3)-linked subunits in these *Acacia* gums remains to be investigated; ionic association between acid groups, for example, is not a major factor here[24].

ACKNOWLEDGMENTS

The financial support of the South African Council for Scientific and Industrial Research and the University of Cape Town (Staff Research Fund) is gratefully acknowledged. We thank Dr. D. M. W. Anderson (Edinburgh) and Dr. E. G. H. Oliver (Botanical Research Unit, Stellenbosch) for providing gum specimens, and Professor E. A. C. L. E. Schelpe (Bolus Herbarium, University of Cape Town) for classifying some of the specimens.

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